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## **Absence of SARS-CoV-2 in Semen of a COVID-19 Patient Cohort**

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## ABSTRACT

**Background:** Since SARS-CoV-2 infection was first identified in December 2019, the novel coronavirus-induced pneumonia COVID-19 spread rapidly and triggered a global pandemic. Recent bioinformatics evidence suggests that angiotensin converting enzyme 2—the main cell entry target of SARS-CoV-2—is predominantly enriched in spermatogonia, Leydig and Sertoli cells, which suggests the potential vulnerability of the male reproductive system to SARS-CoV-2 infection.

**Objectives:** To identify SARS-CoV-2 RNA in seminal plasma and to determine semen characteristics from male patients in the acute and recovery phases of infection.

**Methods:** From February 26 to April 2, 2020, 23 male patients with COVID-19 were recruited. The clinical characteristics, laboratory findings and chest computed tomography scans of all patients were recorded in detail. We also investigated semen characteristics and the viral RNA load in semen from these patients in the acute and recovery phases of SARS-CoV-2 infection using approved methods.

**Results:** The age range of the 23 patients was 20–62 years. All patients tested negative for SARS-CoV-2 RNA in semen specimens. Among them, the virus had been cleared in 11 patients, as they tested negative. The remaining 12 patients tested negative for SARS-CoV-2 RNA in semen samples, but were positive in sputum and fecal specimens. The median interval from diagnosis to providing semen samples was 32 days, when total sperm counts, total motile sperm counts and sperm morphology of the patients were within normal ranges.

**Discussion and Conclusion:** In this cohort of patients with a recent infection or recovering from

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COVID-19, there was no SARS-CoV-2 RNA detected in semen samples, which indicates the unlikely possibility of sexual transmission through semen at about 1 month after first detection.

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## 1 | INTRODUCTION

Since the first outbreak of SARS-CoV-2 coronavirus infections causing COVID-19 in Wuhan, China, in December 2019, this form of pneumonia spread rapidly and triggered a global pandemic.<sup>1,2</sup> Up to 10:00 am on May 28, 2020, the total confirmed cases of COVID-19 worldwide had reached 5,690,951 with 355,615 deaths, according to the data from CSSE at Johns Hopkins University (<https://coronavirus.jhu.edu/map.html>). With this rapid increase, more than 188 countries have been affected, especially the United States, Brazil, Russia, United Kingdom, Spain, and Italy. It is critically important to identify the transmission routes of COVID-19 and help reduce its spread.

Respiratory droplets and close contact are the two main transmission routes of SARS-CoV-2.<sup>3,4</sup> Additionally, there is evidence of SARS-CoV-2 in the feces, urine and tears.<sup>5-7</sup> Angiotensin converting enzyme 2 (ACE2) is considered as the major receptor molecule of SARS-CoV-2 for binding and entry into host cells.<sup>8</sup> Theoretically, any cells expressing ACE2 might be susceptible to SARS-CoV-2 infection. Based on the Human Protein Atlas portal, an online database (<https://www.proteinatlas.org>), testes show the highest expression level of ACE2 in humans.<sup>9</sup> Furthermore, according to recent bioinformatic evidence using single cell RNA sequencing profiling, Wang et al. also reported that ACE2 is predominantly enriched in spermatogonia, Leydig and Sertoli cells.<sup>10</sup> All the findings suggest the potential risk of the male reproductive system to be vulnerable to SARS-CoV-2 infection.

During the period of active viremia, viruses might be shed into the male genital tract via an imperfect blood–testis barrier.<sup>2</sup> In fact, a wide range of viruses, such as Zika, Ebola, Influenza, Epstein Barr viruses that result in viremia can be detected in human semen.<sup>11,12</sup> Moreover, specific male organs or cells could act as mid-term or long-term reservoirs for the viruses once infected. However, there is no definitive clinical evidence about whether SARS-CoV-2 infection can be detected in human semen and the possible consequences of such infection on semen parameters in men are unknown to date.

Here, we aimed to identify viral RNA in seminal plasma and to determine semen

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characteristics respectively from male patients during the acute and recovery phases of SARS-CoV-2 infection. We aimed to evaluate whether SARS-CoV-2 can be detected in human semen and its possible effect on semen parameters during infection, and thus pose a potential risk for sexual transmission.

## **2 | METHODS**

### **2.1 | Study design and patients**

We recruited 23 male patients with COVID-19 from February 26 to April 2, 2020 in Shandong, P. R. China. All cases were laboratory-confirmed as SARS-CoV-2 positive using quantitative reverse transcription polymerase chain reaction (RT-qPCR) amplification on pharyngeal swab specimens. The diagnosis of COVID-19 was determined according to the New Coronavirus Pneumonia Prevention and Control Program (7th edition) published by the National Health Commission of China. Written consent was obtained from each enrolled patient. This study was reviewed and approved by the Medical Ethical Committee of Shandong Provincial Hospital (SWYX: NO.2020-043). The trial has been registered in the Chinese Clinical Trial Registry (ChiCTR2000031779).

### **2.2 | Data collection**

The patients' medical characteristics including demographic data, medical history, potential comorbidities, symptoms, signs, chest computer tomography (CT) scans, laboratory results and treatment measures were recorded. All information was obtained and curated using a standardized data collection form. A team of medical specialists reviewed the data collection forms to check the data. Different clinical categories were defined for participants in the study according to the New Coronavirus Pneumonia Prevention and Control Program (7th edition) published by the National Health Commission of China, including mild, moderate, severe, and critical types.

Semen samples were obtained by masturbation during the COVID-19 recovery phase considering the patient's physiological and psychological acceptance. To avoid virus contamination from other non-semen sources, all subjects were required to wash their hands and penis before masturbation and ejaculate into a sterile container according to the World Health

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Organization (WHO) guidelines for semen analysis. Twenty-one semen samples were obtained by masturbation after the recommended 3–6 days abstinence period and processed within 1 h of ejaculation for analysis. Two samples (Patient 4 and Patient 18) were excluded from analysis with a volume of <0.5 mL and non-liquefaction of semen. Semen analysis was done according to WHO guidelines. Pharyngeal swab specimens were tested on the day of admission to the trial, and sputum and fecal specimens were collected every few days thereafter for virus testing.

All samples were tested for SARS-CoV-2 using specific kits (Huirui Biotechnology, Shanghai, P. R. China) as recommended by the Chinese Center for Disease Control and Prevention (CDC). The criteria for the confirmed diagnosis of SARS-CoV-2 were that at least one site was amplified to be positive for the nucleocapsid protein (NP) gene and its open reading frame (ORF) during the RT-qPCR assay in nasopharyngeal swab/sputum/semen testing. Anti-SARS-CoV-2 virus IgG and IgM antibodies were detected using commercial colloidal gold test kits (Wondfo Biotechnology, Guangzhou, P. R. China) as recommended by the CDC.

### **2.3 | Statistical analysis**

Categorical variables are described as frequencies and percentages. Continuous measurements are presented as the mean  $\pm$  standard deviation (SD) if they were normally distributed, or as the median and interquartile range (IQR) if they were not. All statistical analyses were performed using IBM SPSS statistics (v. 20.0; IBM Corp., Armonk, NY, USA).

## **3 | RESULTS**

Twenty-three patients with COVID-19 were enrolled in this study, with an age range of 20–62 y, (Table 1). Among 23 men with COVID-19, 18 (78%) were diagnosed as having the mild type, and five (22%) as having the moderate type, but none developed severe or critical pneumonia. Treatments included therapy with arbidol, lopinavir and ritonavir, traditional Chinese medicine, interferon- $\alpha$  inhalation, oxygen ventilation, and antibiotics. The clinical characteristics of the patients are presented in Table 1. All underwent chest CT scans on admission. The most common patterns were patchy shadowing in nine (39%), emphysema in three (13%), and ground-glass opacity in three (13%). Two patients (9%) showed old tuberculosis lesions in their lungs. The remaining eight (35%) patients showed normal CT scans. Up to April 2, 2020, a total

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of 16 patients (70%) had been discharged while the remaining seven were kept in hospital for further observation.

RNA analysis and antibody tests for SARS-CoV-2 among all specimens was conducted during hospitalization. As shown in Table 2, all patients tested positive for SARS-CoV-2 RNA in pharyngeal swab specimens on admission. However, all of them tested negative in semen specimens. Among these, the virus had been cleared based on pharyngeal swabs, sputum specimens, and fecal specimens in 11 patients when they tested negative for SARS-CoV-2 RNA in semen samples. The remaining 12 patients tested negative for SARS-CoV-2 RNA in semen samples, but were still positive in sputum or fecal specimens. Among the 23 patients, 22 developed an immune response against SARS-CoV-2, while IgM and IgG antibodies were negative in one patient. Semen characteristics are listed in Table 2. The median duration from diagnosis to producing semen samples was 32 days, when total sperm counts, total motile sperm counts and sperm morphology distributions were within normal ranges.

#### 4 | DISCUSSION

Accumulating evidence suggests that respiratory droplets and intimate contact are the two main transmission routes of SARS-CoV-2.<sup>1,3,4,13</sup> Aerosol transmission and fecal-oral transmission routes are also possible.<sup>14,15</sup> Vertical transmission and sexual transmissions among female patients have not been verified.<sup>16,17</sup> Additionally, asymptomatic infected persons, with the long incubation period for this virus, are potential sources of infection.<sup>18</sup> Remarkably, the potential routes of transmission of SARS-CoV-2 are still worth exploring.

It is known that a broad range of viral families, including Zika Ebola, human immunodeficiency virus (HIV), and Hepatitis viruses B/C can be transmitted into semen and result in sexual transmission.<sup>12</sup> Additionally, many viruses such as mumps virus, influenza, HIV, and Zika virus, can induce orchitis and cause male infertility.<sup>19</sup> The human male reproductive system is vulnerable to virus infection because the blood–testis barrier is not able to prevent viral entry completely.<sup>2</sup> Moreover, not all antiviral therapeutics are able to cross the blood–testis barrier, so viruses can persist in the semen despite systemic clearance. Furthermore, sexual transmission is a

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major concern, particularly for pregnant women and couples wishing to conceive. Therefore, the investigation of virus detection and persistence in semen is useful for clinical and public health reasons, particular for viruses that lead to high mortality or morbidity rates.

Here, we collected semen samples from 23 patients in the acute and recovery phases of COVID-19. All samples tested negative for SARS-CoV-2 RNA using RT-qPCR assays, and the semen characteristics were all within normal ranges, which indicates the lack of a sexual transmission route for this coronavirus. Therefore, from our results, there is no evidence of the virus being expressed in human semen samples, which indicates that the testis might be not a target organ for SARS-CoV-2, and the virus will not appear in the semen regardless of the sputum or fecal results. Consistent with our results, a previous study showed that SARS coronaviruses were undetectable in the testis,<sup>20</sup> despite orchitis being a potential complication of SARS, primarily based on the systemic inflammatory response and virus-induced autoimmune response among severely affected patients.<sup>21</sup> Two other studies have confirmed the absence of SARS-CoV-2 in semen. Song et al. examined the semen of 13 SARS-CoV-2-positive patients between January 31 and March 14, 2020 using RT-qPCR or anti-SARS-CoV-2 antibodies (both IgM and IgG) in serum by colloidal gold-based immunoassays. All semen samples were negative for SARS-CoV-2 RNA and testicular samples from one deceased patient were negative.<sup>22</sup> In another study, a 31-year-old man from Italy tested positive for SARS-CoV-2 through a pharyngeal swab; but his semen and urine samples were negative for SARS-CoV-2 RNA.<sup>23</sup> Likewise, Pan et al. investigated single semen samples from 34 Chinese men using SARS-CoV-2 RNA amplification, which confirms again the absence of the virus in all samples.<sup>24</sup>

In contrast to the above findings, semen samples from 38 patients in Shangqiu, P. R. China, were tested for the presence of COVID-19 virus. Twenty-three participants had achieved clinical recovery and 15 participants were still in the acute stage of infection. Six patients had positive results for SARS-CoV-2 in the semen, including 4/15 patients at the acute stage of infection and 2/23 patients who were in the recovery stage.<sup>25</sup> However, this study did not describe the semen collection or analysis in detail, nor was there evidence of SARS-CoV-2 in the urine of these patients, so the possibility of viral contamination from non-semen sources could not be excluded



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completely.<sup>26</sup>

Compared with the aforementioned studies, we found that these men with COVID-19 had a normal range of semen characteristics about 32 days after the first detected infection. Among them, the virus had been cleared in 10 when they underwent semen analysis. The remaining 11 patients had moved into the recovery phase but were still viral RNA positive in sputum or fecal specimens. Considering the duration of human spermatogenesis, SARS-CoV-2 infection is unlikely to cause semen quality to decline at the onset. However, our results demonstrated that after an average of 32 days after diagnosis, semen parameters were still within normal ranges. Because most of these patients have entered the recovery period of COVID-19, we anticipate that subsequent semen parameters are likely to be normal, despite potential inflammatory responses or virus-induced autoimmune response in the testis.

Our study had several limitations. First, because of the small sample size, only 18 mild and five moderate cases of COVID-19 were included, and the lack of severe and critical cases could have affected the power of our statistical analysis. Second, a longitudinal assessment with repeated RNA detection and semen parameters with appropriate time intervals is necessary. Third, no testicular biopsies were collected, which would give conclusive evidence for any injury caused by SARS-CoV-2 infection. Nevertheless, the data in our study revealed the absence of SARS-CoV-2 in human semen and indicated that the testes and male genital tract are able to avoid infection with this coronavirus.

## **5| CONCLUSION**

In this cohort of patients with a recent infection or recovering from COVID-19, there was no SARS-CoV-2 RNA detected in semen samples, which indicates the unlikely possibility of sexual transmission through semen at about 1 month after first detection.

## **6| ACKNOWLEDGMENTS**

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## 7| CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Table 1: Clinical characteristics of 23 male patients with COVID-19

| COVID-19 Patients, n=23 |                              |               |
|-------------------------|------------------------------|---------------|
| Age, Mean±SD years      |                              | 41.04±11.56   |
| Range                   |                              |               |
|                         | <30                          | (8.70%)2/23   |
|                         | 30~39                        | (30.43%)7/23  |
|                         | 40~49                        | (34.78%)8/23  |
|                         | ≥50                          | (26.09%)6/23  |
| First symptom           |                              |               |
|                         | Fever                        | (30.43%)7/23  |
|                         | Cough                        | (17.39%)4/23  |
|                         | Sore throat                  | (17.39%)4/23  |
|                         | Diarrhea                     | (8.70%)2/23   |
|                         | Chest pain                   | (8.70%)2/23   |
|                         | Anorexia                     | (17.39%)4/23  |
|                         | Rash                         | (8.70%)2/23   |
| Abnormal Chest CT (%)   |                              | (65.22%)15/23 |
|                         | Patchy shadowing             | (39.13%)9/23  |
|                         | Emphysema                    | (13.04%)3/23  |
|                         | Old tuberculosis             | (8.70%)2/23   |
|                         | Ground-glass opacity         | (13.04%)3/23  |
| Clinical group          |                              |               |
|                         | Mild                         | (78.26%)18/23 |
|                         | Moderate                     | (21.74%)5/23  |
|                         | Severe                       | 0             |
|                         | Critical                     | 0             |
| Treatment               |                              |               |
|                         | Arbidol                      | (100%)23/23   |
|                         | Lopinavir and Ritonavir      | (100%)23/23   |
|                         | Interferon-α inhalation      | (69.57%)16/23 |
|                         | Oxygen therapy               | (65.22%)15/23 |
|                         | Traditional Chinese medicine | (100%)23/23   |
|                         | Antibiotic treatment         | (13.04%)3/23  |
|                         | Chloroquine phosphate        | (8.70%)2/23   |
|                         | Immunoglobulin               | (8.70%)2/23   |

Different clinical categories were defined for participants in the study according to the New Coronavirus Pneumonia Prevention and Control Program (7th edition) including mild type, the moderate type, the severe type, and the critical type. COVID-19: coronavirus disease 2019.

Table 2: Laboratory characteristics for adult males with confirmed COVID-19

| ID | Admission day | March 31, 2020     |        |            |       |                 |                 |                  |                                |          |            |           |  |
|----|---------------|--------------------|--------|------------|-------|-----------------|-----------------|------------------|--------------------------------|----------|------------|-----------|--|
|    |               | Specimens for      |        | SARS-CoV-2 |       | Immune antibody |                 | Semen parameters |                                |          |            |           |  |
|    |               | Pharyngeal<br>swab | Sputum | Feces      | Semen | IgM<br>antibody | IgG<br>antibody | Semen            | Sperm                          | Motility | Motility   | Normal    | Time interval from diagnosis<br>to semen samples acquired(d) |
|    |               |                    |        |            |       |                 |                 | volume (mL)      | concentration<br>(million /mL) | (PR, %)  | (PR+NP, %) | forms (%) |  |
|    |               |                    |        |            |       |                 |                 |                  |                                |          |            |           |  |
| 1  | +             | -                  | -      | -          | -     | +               | 1.2             | 83               | 50                             | 70       | 20         | 32        |  |
| 2  | +             | -                  | -      | -          | -     | +               | 1.5             | 106              | 10                             | 30       | 14         | 33        |  |
| 3  | +             | -                  | -      | -          | +     | +               | 4               | 2                | 30                             | 50       | 12         | 32        |  |
| 4  | +             | -                  | -      | -          | -     | +               | <0.5            |                  |                                |          |            | 32        |  |
| 5  | +             | -                  | -      | -          | -     | +               | 2.7             | 57               | 60                             | 70       | 14         | 26        |  |
| 6  | +             | -                  | -      | -          | -     | +               | 0.9             | 132              | 60                             | 70       | 24         | 33        |  |
| 7  | +             | -                  | -      | -          | -     | +               | 1.1             | 44               | 55                             | 85       | 14         | 27        |  |
| 8  | +             | -                  | -      | -          | -     | +               | 2.2             | 252              | 50                             | 65       | 8          | 28        |  |
| 9  | +             | -                  | +      | -          | -     | -               | 3               | 47               | 15                             | 25       | 10         | 26        |  |
| 10 | +             | -                  | -      | -          | -     | +               | 1.5             | 136              | 60                             | 80       | 22         | 31        |  |
| 11 | +             | -                  | -      | -          | -     | +               | 1               | 95               | 50                             | 60       | 6          | 34        |  |
| 12 | +             | -                  | -      | -          | -     | +               | 1.6             | 242              | 35                             | 55       | 12         | 33        |  |
| 13 | +             | -                  | +      | -          | +     | +               | 2.4             | 56               | 60                             | 70       | 30         | 32        |  |
| 14 | +             | -                  | +      | -          | +     | +               | 2               | 56               | 70                             | 80       | 24         | 26        |  |
| 15 | +             | -                  | +      | -          | -     | +               | 4.6             | 184              | 40                             | 60       | 16         | 33        |  |
| 16 | +             | -                  | +      | -          | -     | +               | 1               | 75               | 70                             | 80       | 28         | 34        |  |
| 17 | +             | -                  | +      | -          | -     | +               | 4               | 55               | 70                             | 80       | 12         | 32        |  |
| 18 | +             | -                  | +      | -          | +     | +               | <0.5            |                  |                                |          |            | 34        |  |
| 19 | +             | -                  | +      | -          | +     | +               | 2.7             | 175              | 40                             | 60       | 22         | 33        |  |
| 20 | +             | -                  | +      | -          | +     | +               | 3               | 122              | 30                             | 40       | 22         | 27        |  |
| 21 | +             | -                  | +      | -          | +     | +               | 2.7             | 98               | 50                             | 60       | 16         | 34        |  |
| 22 | +             | -                  | +      | -          | +     | +               | 3.2             | 230              | 42                             | 72       | 14         | 32        |  |
| 23 | +             | +                  | -      | -          | +     | +               | 2.3             | 94               | 40                             | 60       | 20         | 33        |  |

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; PR: progressive (motility); NP: Non-progressive (motility). Two semen samples (Patient 4 and Patient 18) were excluded for semen parameter analysis due to the semen volume less than 0.5ml and non-liquefaction of semen. Data are presented as Median (IQR).